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M E. Davis
West Virginia University

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Effect of Chloroacetic Acids on the Kidneys

by Mary E. Davis*

The effects of dichloroacetate (DCA) and trichloroacetate (TCA) administered in drinking water were studied. At high concentrations of either compound, weight loss, or failure to gain weight, was observed. Food consumption was also decreased; both effects were attributed to decreased water consumption. Renal phosphate-dependent glutaminase activity was increased at the highest concentration, and urinary ammonia was also increased. These changes indicated renal adaptation to an acid load. DCA, in pharmacological doses, impairs gluconeogenesis from lactate in part by decreasing lactate availability. Similar tendencies were observed in the present studies; however, female rats showed a biphasic response. At lower DCA concentrations, tissue lactate and plasma glucose concentrations were increased, whereas at higher concentrations of DCA, the expected decreases were observed.

Introduction

The effects of high, pharmacological doses of dichloroacetate (DCA) have been elucidated in recent years and are reviewed by Crabb and co-workers (1). DCA activates the pyruvate dehydrogenase complex, resulting in decreased synthesis of glucose from lactate. In addition, DCA is metabolized in the body, producing glyoxylate and oxalate. These compounds inhibit other enzymes, including pyruvate carboxylase and pyruvate kinase. These additional metabolic derangements result in excessive production of ketone bodies (2). DCA also decreases blood cholesterol concentrations and has been used to treat hypercholesterolemia, but its usefulness for long-term therapy is limited by the development of peripheral polyneuropathy (3). DCA may still be a useful treatment for acute lactic acidosis.

The toxicity of trichloroacetate (TCA) has been studied much less. TCA is used extensively for its ability to precipitate proteins, primarily in biological research, but also in dermatology for chemical dermabrasion. The studies reported here focus on drinking water exposure to DCA and TCA, in an attempt to determine if effects shown at higher doses, and in *in vitro* studies, might occur when DCA or TCA is present in drinking water.

Methods

Male and female rats of Sprague-Dawley ancestry, supplied by Hilltop Farms, were used in all experiments. The animals were maintained in the central animal facilities on lab chow (Wayne Lab Blox).

DCA was administered in drinking water for 14 days. The concentrations of DCA used (0, 0.03, 0.125, 0.5, and 1.875 g/L) were chosen to yield target doses of (0, 10,

40, 150, and 600 mg/kg/day). Initially a higher dose, 7.5 g/L, was included. The solutions were made up in reagent-grade water. During DCA administration, the animals were maintained individually in stainless-steel metabolism cages; body weight and food and water consumption were measured daily. Urine was collected on days 7 and 14; volume, osmolality, and ammonia were measured. At the end of the treatment, the rats were etherized, blood samples were taken from the abdominal aorta, and pieces of liver and kidney were taken and stored for analyses. Samples to be used for lactate and pyruvate assays were rapidly frozen using clamps at liquid nitrogen temperature. Plasma was separated and stored frozen for glucose analysis.

Lactate and pyruvate were assayed by reduction and oxidation, respectively, of nicotinamide adenine dinucleotide (reduced) (NADH) in the presence of lactate dehydrogenase (4,5). Ammonia was measured as utilization of NADH in the synthesis of glutamate from 2-oxoglutarate, catalyzed by glutamate dehydrogenase (6). Hippurate was measured colorimetrically based on its reaction with dimethylaminobenzaldehyde (7). Glucose was measured colorimetrically with Sigma reagents (kit 115) based on the hexokinase and glucose-6-phosphatase reactions. Phosphate-dependent glutaminase (PDG) and phosphate-independent glutaminase (PIG) activities were measured as described by Curthoys and Lowry (8), except that the glutamate produced was measured using Witt's method (9), which uses the nicotinamide adenine dinucleotide (NAD) analog, 3-acetylpyridine, to favor the reverse direction of the glutamate dehydrogenase reaction. Osmolality was measured by freezing point depression, using an Osmette S osmometer.

For the first TCA study, the compound was administered by gavage, in doses of 30 and 300 mg/kg, daily for 7 days. In the next study, TCA was administered

*Department of Pharmacology and Toxicology, West Virginia University Medical Center, Morgantown, WV 26506.

Table 1. Body weight during dichloroacetate administration in drinking water to male rats.

	Body weight, g					
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L	DCA 7.50 g/L
Day 0	335 ^a	362	321	334	324	294
SEM	13	5	11	18	9	5
Day 7	346	365	322	340	313	253
SEM	10	9	13	17	17	3
Day 14	365	371	332	355	324	277
SEM	8	8	12	15	13	3

^aResults are expressed as grams of body weight and are shown as mean \pm SEM of five rats, except for $n = 2$ for 7.50 g/L.

in drinking water at concentrations of 0.3 and 3 g/L to yield target doses of 24 and 240 mg/kg/day. These rats were maintained in plastic group cages and were placed in metabolism cages on days 14 and 21 for urine sampling.

All results are expressed as mean \pm standard error of the mean. Data were analyzed by ANOVA using the model appropriate to the experimental design. Statistical analyses were done using SAS software from SAS Institute Inc., Cary, NC. The criterion for significance was $p < 0.05$.

Results and Discussion

Results for body weight on days 0, 7, and 14 are shown in Table 1. The high dose (7.5 g/L) caused weight loss (17% of starting weight by day 4) and was used only in the first group of males. The effects of dose and day and the interaction between dose and day are significant. The food consumption data (not shown) mirror the body-weight data. Results for female rats are summarized in Table 2; the 7.5 concentration was not used. The treatment effect was not significant for females. The female rats on the highest concentration lost weight, approximately 5% of their starting weight, over the first 5 days, but then they regained this loss. During this time, their food consumption was also slightly lower

Table 2. Body weight during dichloroacetate administration to female rats.

	Body weight, g				
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L
Day 0	254 ^a	256	251	249	258
SEM	9	12	11	10	9
Day 7	253	254	259	249	249
SEM	10	11	12	9	7
Day 14	262	262	265	254	256
SEM	9	10	14	9	6

^aResults are expressed as grams of body weight and are shown as mean \pm SEM of six rats, except $n = 7$ for 1.88 g/L.

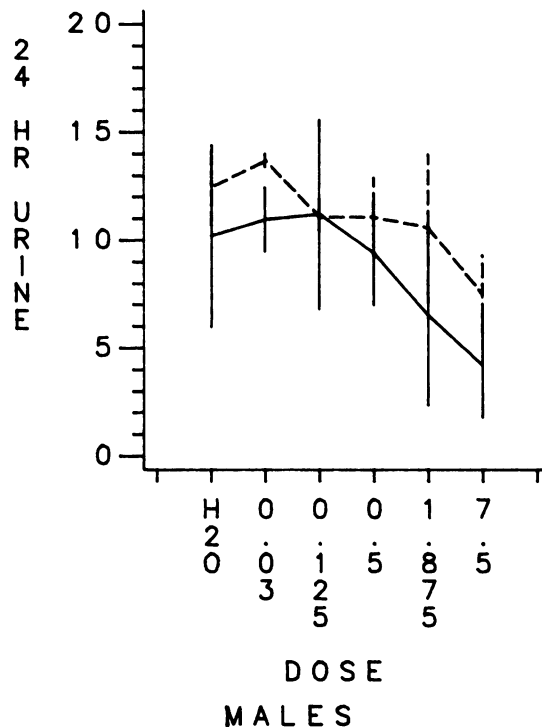


FIGURE 1. Effect of dichloroacetate on urine output. Male rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed in mL and are shown as mean \pm SE of five rats, except $n = 2$ for the 7.5 g/L concentration.

(17 g for controls vs. 12 g for the 1.8 g/L DCA group). Water loss from the bottle was monitored in all groups as an approximation of water consumption. The high-dose groups consumed less water than did the others. Lower water consumption would account for the decreased food consumption, because rats generally will not consume dry chow if water is not available.

Results for urine output on days 7 and 14 are shown in Figures 1 (males) and 2 (females). Urine volume was not significantly affected by DCA treatment in either sex; however, at higher DCA concentrations urine volumes were somewhat decreased on day 7, and less so on day 14. This finding is consistent with the pattern of reduced water consumption in these groups. Osmolality data are shown in Figures 3 and 4. For males, the effect of DCA treatment and the interaction between treatment and day of treatment were significant; for females no significant effects were detected. Osmolality and volume were inversely related, that is, reductions of urine volume were countered by elevations of urine osmolality. Taken together, these results indicate that the ability to concentrate urine is not impaired by exposure to DCA.

Excretion of ammonia in urine was measured as one index of renal adaptation to the acid load. Results for day 14 are summarized, for both males and females, in Table 3. The results are expressed as total ammonia excreted, on day 14, to account for differences of urine volume, and the results are normalized for body weight.

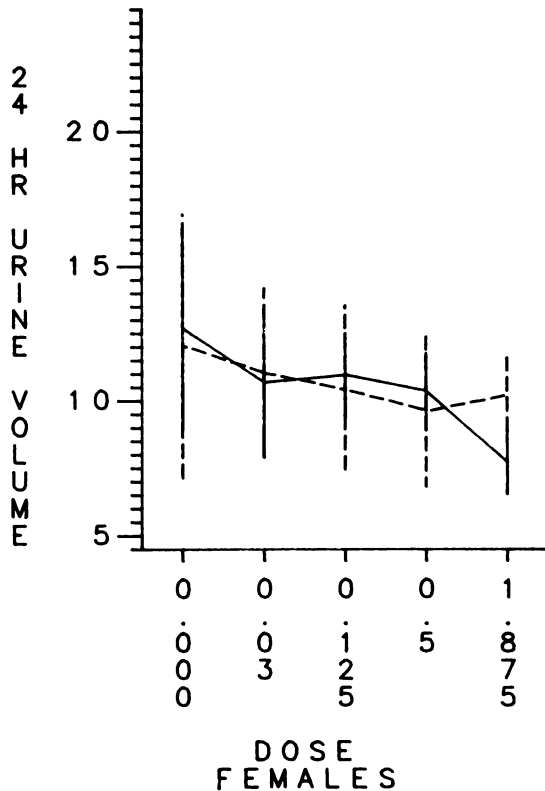


FIGURE 2. Effect of dichloroacetate on urine output in female rats. Rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed in mL and are shown as mean \pm SE of six rats.

The effect of DCA treatment was significant for the males, but not for the females. The males showed a normal concentration-response relationship; however, for females, a biphasic relationship was observed. Ammonia is produced in the kidneys by the enzymes phosphate dependent and independent glutaminase. PDG is activated by acidosis (8); increased activity of this glutaminase increases excretion of ammonium ion. PIG is identical to glutamyltranspeptidase (10); during acidosis, the glutaminase activity predominates. Decreased pH of tubular urine favors the glutaminase reaction. Hippurate, produced during acidosis, activates PIG (11) by binding to the amino acid site (12). Results for PIG and PDG activities in kidneys from female rats are shown in Table 4. The effect of DCA treatment was significant for PDG, but not for PIG. Furthermore, the pattern of the effect is similar to that of ammonia excretion: a decrease at low DCA concentrations and an increase at high DCA concentrations. Results for hippurate excretion are shown in Figures 5 (males) and 6 (females). The effect of DCA administration was not significant for either sex, on either day 7 or 14. These results suggest that renal compensation for the acid load posed by DCA administration is accomplished primarily by activation of PDG. Hippurate production was not

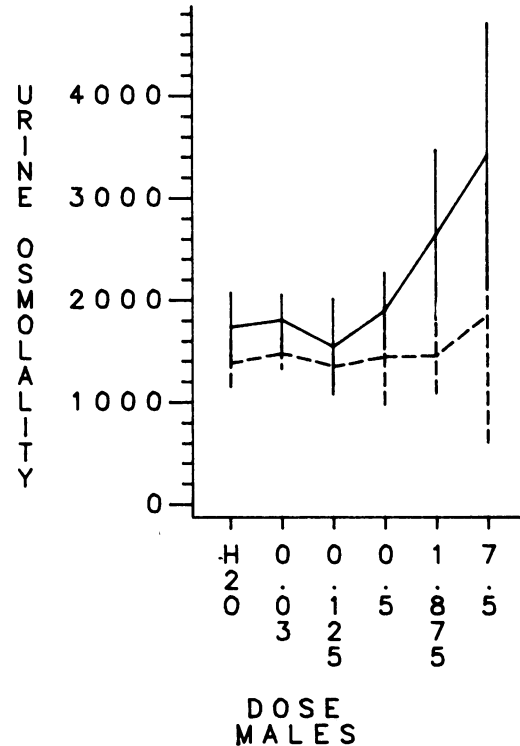


FIGURE 3. Urine osmolality in male rats. Male rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed as mOsmoles/kg water and are shown as mean \pm SE of five rats, except $n = 2$ for the 7.5 g/L concentration.

increased, and so was not able to activate the glutaminase activity of γ -glutamyltranspeptidase.

DCA treatment has been reported to activate the pyruvate dehydrogenase complex in liver and kidney, among other tissues. Oxalate, a metabolite of DCA, inhibits pyruvate kinase (which catalyzes the step in the glycolytic pathway from phosphoenol pyruvate to pyruvate) and pyruvate carboxylase (which converts pyruvate into oxaloacetate). The net effect of these metabolic derangements is to decrease the availability of gluconeogenic substrates, decrease glucose synthesis, and increase production of acetoacetate metabolites (1). Administration of high doses of DCA decreases plasma concentrations of glucose, lactate, and pyruvate. Tissue concentrations of lactate and pyruvate, for female rats, are shown in Tables 5 (liver) and 6 (kidney). Results were similar for both tissues, and the effect of DCA treatment was not significant for either. The expected decrease of liver lactate content was observed, suggesting that the high DCA concentration approaches a minimally effective dose. The results for liver lactate are similar to the biphasic response seen with females for ammonia excretion and renal glutaminase activity. Preliminary results indicate that renal and hepatic lactate contents are decreased by DCA administration in male rats. Plasma glucose results are shown for both males and females in Table 7. The effect of DCA treat-

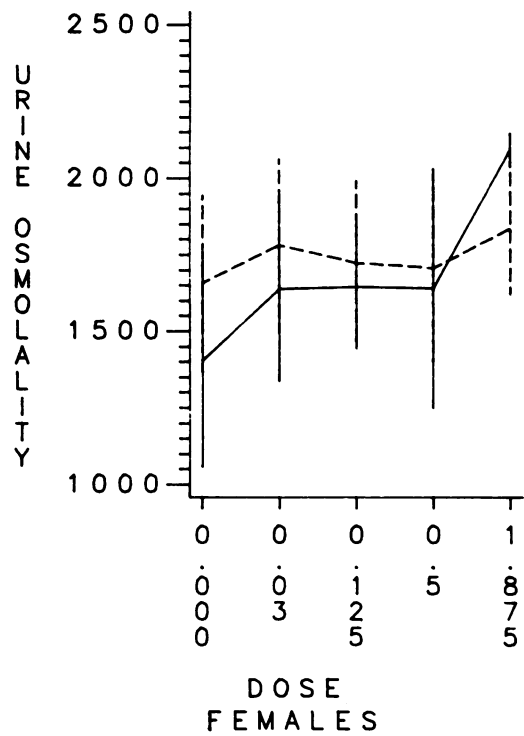


FIGURE 4. Urine osmolality in female rats. Rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed as mOsmole/kg water and are shown as mean \pm SE of six rats.

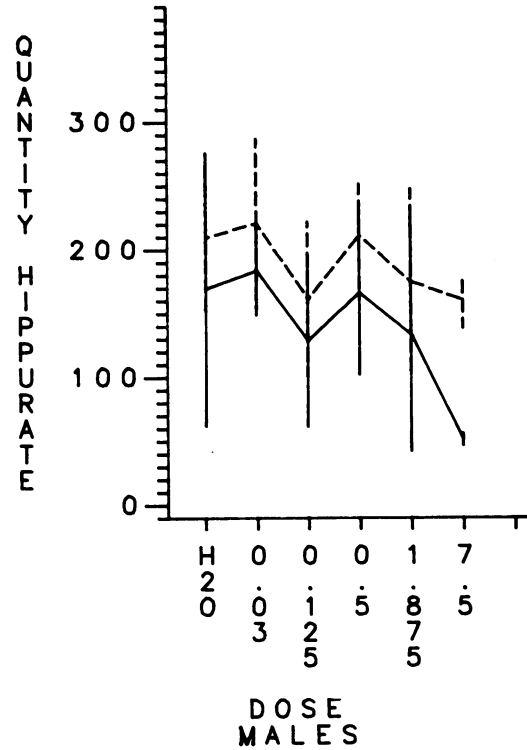


FIGURE 5. Hippurate excretion in male rats. Male rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed as μ g/day and are shown as mean \pm SE of five rats, except $n = 2$ for the 7.5 g/L concentration.

Table 3. Ammonia excretion in urine on day 14.

Sex	NH ₄ ⁺ μ mole/g body weight					
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L	DCA 7.5 g/L
Males	0.232	0.263	0.694	0.607	0.751	1.186
SEM	0.026	0.096	0.239	0.109	0.277	0.125
n	5	5	5	5	5	2
Females	0.517	0.275	0.365	0.710	0.740	nd ^a
SEM	0.084	0.047	0.094	0.277	0.181	
n	6	6	6	6	7	

^aNot done.

Table 4. Renal glutaminase activities for female rats.

	Glutamate formed, μ mole/min/g kidney weight				
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L
PIG	7.17	5.44	5.29	5.93	5.37
SEM	0.75	0.84	0.88	0.83	1.91
n	5	4	5	5	5
PDG	5.74	4.86	4.51	6.78	7.21
SEM	0.57	0.36	0.82	0.45	0.17
n	5	4	5	5	5

ment was not significant for either sex. As with the lactate results, the values for the high dose reflect the expected decrease; furthermore, the females exhibited a biphasic pattern. Further studies are necessary to confirm this effect and to elucidate the mechanism for the biphasic response.

The effects of TCA administration by gavage are shown in Figure 7 for body weight and Figure 8 for food consumption. The low dose, 30 mg/kg, did not alter either body weight or food consumption; the rats gained weight parallel to the controls, and in general the low-dose group appeared to be normal. The high-dose (300 mg/kg) group did not fare as well. One rat died on the second day, and the three survivors, which were hyperventilating and cyanotic, were sacrificed on the third day. The lungs of these rats appeared normal by gross observation, and the hyperventilation was very likely respiratory compensation for the acid load (Kussmaul breathing). The initial study on TCA administration in water was carried out over 3 weeks, with two concentrations of TCA. Results for body weight are summarized in Table 8. The effect of TCA administration was significant. Although the groups started out at the same weights, the high-concentration TCA group did not gain weight as rapidly as the others. Food consumption was not measured in this study; however, it is very likely

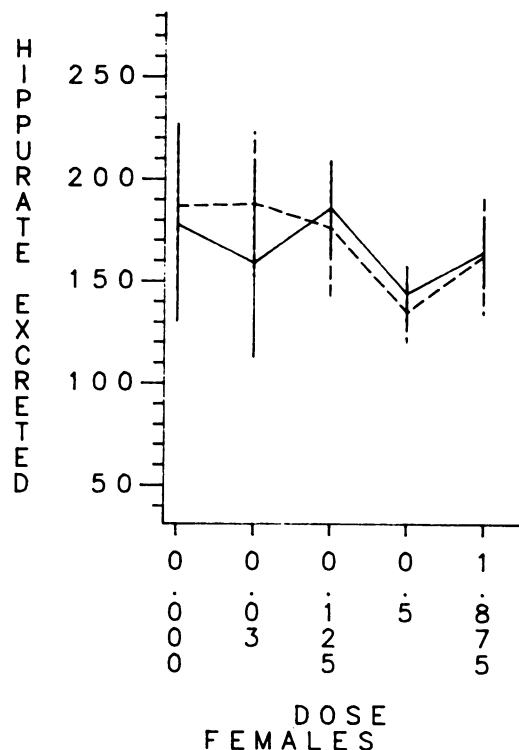


FIGURE 6. Hippurate excretion in female rats. Rats were given water containing DCA in the concentrations (g/L) indicated. Urine was collected on (—) day 7 and (---) day 14. Results are expressed as $\mu\text{g/day}$ and are shown as mean \pm SE of six rats.

that it was decreased in this group. The results for urine volume and osmolality are summarized in Table 9. The effects are not significant; however, the group that received the high concentration consistently had lower urine volumes and higher osmolalities. In the DCA studies, results of this type were associated with decreased water consumption; it is very likely that water consumption was decreased in the high-concentration TCA group. The results for osmolality and urine indicate that TCA did not impair urine-concentrating ability.

Table 5. Liver lactate and pyruvate from female rats.

	nmole/g wet weight				
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L
Lactate	1550	1260	1860	950	970
SEM	36	100	330	280	100
n	6	6	5	6	7
Pyruvate	15	15	12	11	12
SEM	4	9	6	5	7
n	5	4	5	5	5
Ratio	120	40	8	20	25
SEM	20	0	3	1	11
n	5	2	2	3	5

Table 6. Kidney lactate and pyruvate from female rats.

	nmole/g wet weight				
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L
Lactate	1300	1240	1320	1270	1060
SEM	200	90	210	260	140
n	6	5	6	6	6
Pyruvate	7	11	7	5	7
SEM	1	3	2	1	2
n	6	5	6	6	7
Ratio	370	170	410	210	180
SEM	200	60	240	70	50
n	6	5	6	5	5

Table 7. Effect of DCA on plasma glucose.

	Plasma glucose, mg/100 mL				
	DCA 0.00 g/L	DCA 0.03 g/L	DCA 0.13 g/L	DCA 0.50 g/L	DCA 1.88 g/L
Females	190	187	212	174	173
SEM	14	12	27	14	11
n	6	4	3	6	3
Males	188	189	194	190	168
SEM	11	12	14	6	8
n	5	4	4	5	5

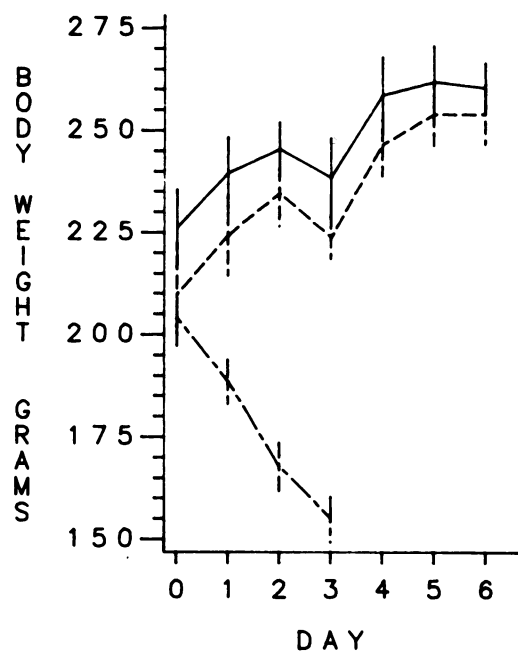


FIGURE 7. Effect of trichloroacetate on body weight. Male rats were given TCA by gavage daily for 7 days: (—) controls; (---) 30 mg/kg/day; (....) 300 mg/kg/day. Results are shown as mean \pm SE of four rats.

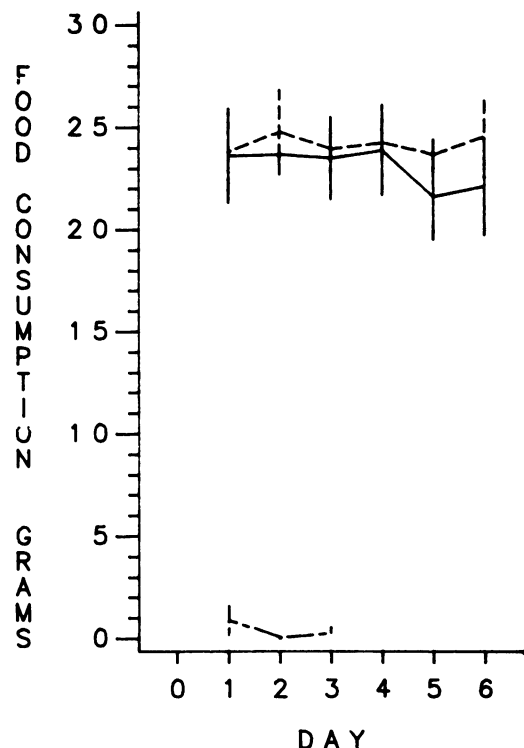


FIGURE 8. Food consumption during trichloroacetic acid administration: (—) controls; (---) 30 mg/kg/day; (....) 300 mg/kg/day. Results are shown as mean \pm SE of four rats.

Table 8. TCA effect on body weight.

DCA conc. g/L	Body weight, g ^a			
	0	7 days	14 days	21 days
0	234	258	303	345
SEM	2	3	3	1
0.3	239	258	305	342
SEM	2	3	2	3
3.0	233	248	295	326
SEM	3	4	1	1

^aResults are expressed as grams of body weight and are shown as mean \pm SEM of four male rats per group.

Table 9. TCA effect on urine volume and osmolality.^a

TCA, g/L	Day 14		Day 21	
	Volume, mL	mOsm	Volume, mL	mOsm
0	9 ^a	1877	8	1832
SEM	1	288	1	215
0.3	7	1724	10	1696
SEM	0	87	1	249
3.0	6	2034	6	1964
SEM	1	119	1	129

^aResults are shown as mean \pm of four male rats per group.

Summary

The studies presented here indicate that subchronic exposure to DCA, in drinking water, can be associated with derangements of intermediary metabolism, especially metabolism of lactate to glucose. Effects on body weight were observed at higher doses and may represent refusal to consume water containing the higher concentrations of DCA or TCA. Exposure to DCA for 14 days in drinking water resulted in changes of ammonia excretion and activity of enzymes of ammoniogenesis that are indicative of renal compensation for an acid load.

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